Enzymatic synthesis of selected amino acid esters of sugars

Synopsis

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Introduction:

Enzymatic synthesis is advantageous compared to chemical synthesis in many ways. Very few reports are available on the synthesis of amino acyl esters of carbohydrates, which involves mainly proteases, protected and activated amino acids and carbohydrates (Maruyama *et al.* 2002; Park *et al*. 1996; 1999; Riva *et al*. 1988). However, there are practically no reports on the synthesis of amino acyl esters of carbohydrates using unprotected and unactivated amino acids and carbohydrates with lipases as catalysts. The present work describes lipases catalysed synthesis of amino acyl esters of few carbohydrates using unprotected and unactivated amino acids and carbohydrates. Amino acyl esters of carbohydrates possess wide variety of applications in food and pharmaceutical industries as sweeteners, surfactants, microcapsules in pharmaceutical preparation, anti viral nucleoside amino acids, antitumor agents and intermediates in the synthesis of biologically active peptides (Krik 1992; Zaks and Dodds 1997). Use of lipases in non-polar solvents for the synthesis of amino acyl esters of carbohydrates offers many advantages like milder conditions, low temperature, stereo and regio selectivity, easy work out procedures, improved product yields, less byproducts and no colouring of products (Klibanov 1986).

Work done:

Chapter **ONE** deals with literature survey on mainly lipase catalysed esterification reactions. Applications of lipase catalysis in different food and pharmaceutical industries are discussed. Factors affecting esterification reaction like nature of substrates, nature of solvents, thermal stability of lipases, water activity and immobilization are discussed. Different strategies in lipase catalysis, like esterification using reverse micelles,

supercritical carbon dioxide and micro oven assisted reactions are presented. Kinetics of lipase catalysis and optimization of reaction parameters using response surface methodology are also described. The chapter ends with a brief description on the scope of the present investigation.

Chapter **TWO** deals with materials and methods. Chemicals employed and their sources are listed. Methods of preparation of L-amino acyl esters of carbohydrates and the other related appropriate aspects of the same are discussed in detail.

Chapter **THREE** describes results from investigations on the synthesis of L-phenylalanyl-D-glucose **24a-e** and L-phenylalanyl-lactose **29a-c** using lipases from *Rhizomucor miehei* (RML), porcine pancreas (PPL) and *Candida rugosa* (CRL). Enzymatic esterification between L-phenylalanine **2** with D-glucose **5** and lactose **11** using RML, PPL and CRL were investigated in terms of incubation period, solvent, enzyme concentrations, substrate concentrations, buffer salts (pH and concentration) and enzyme reusability. Under the experimental conditions employed, all the three lipases exhibited good esterification potentialities. Among the various solvents mixtures employed for the synthesis of L-phenylalanyl-D-glucose **24a-e**, CH_2Cl_2 and DMF (90:10 v/v) was found to be the best. Both PPL and RML showed maximum conversion yields of L-phenylalanyl-D-glucose **24a-e** (98% and 76% of respectively) at 40% (w/w D-glucose) of enzyme employed and CRL showed a maximum conversion of 64% of L-phenylalanyl-lactose **29a-c** at 50% (w/w lactose) enzyme concentration. In presence of buffer salts, conversion yields enhanced by 10% in case of RML and PPL whereas in case of CRL more than 30% esterification enhancement was observed. At higher equivalents of substrates, decrease in conversion yields observed could be due to inhibition at higher

concentrations of L-phenylalanine **2**, D-glucose **5** and lactose **11**. In the synthesis of L-phenylalanyl-D-glucose **24a-e** RML could be reused upto four cycles where as PPL could used only upto two cycles.

Reaction conditions for *Rhizomucor miehei* lipase (RML) catalysed synthesis of L-phenylalanyl-D-glucose **24a-e** using unprotected L-phenylalanine **2** and D-glucose **5** were optimized using Response Surface Methodology (RSM). A Central Composite Rotatable Design (CCRD) was employed involving 32 experiments of five variables (L-phenylalanine concentration in mmol, amount of RML in mg, pH, incubation period in h and buffer concentration in mM) at five levels. A second order polynomial equation was developed in terms of linear, quadratic and cross product terms to study the effects of variables on esterification yields. Surface and contour plots obtained, explained the esterification behaviour clearly. An optimum predicted yield of 1.01 mmol for L-phenylalanyl–D-glucose at 3 mmol L-phenylalanine, 100 mg of RML, 24h incubation period, 0.5 mM (0.5 mL of 0.1M buffer), pH 4.8 acetate buffer was found to agree with 0.97 mmol obtained under these experimental conditions. Validation experiments carried out under random conditions also exhibited good correspondence between predicted and experimental yields.

Chapter **FOUR** describes the syntheses and characterization of L-prolyl **1**, L-phenylalanyl **2**, L-tryptophanyl **3** and L-histidyl **4** esters of carbohydrates (D-glucose **5**, D-galactose **6**, D-mannose **7**, D-fructose **8**, D-arabinose **9**, D-ribose **10**, lactose **11**, maltose **12**, sucrose **13**, D-mannitol **14**, D-sorbitol **15**). Esterification was carried out using lipase from *Candida rugosa* (50% w/w carbohydrate) in 100 mL of CH_2Cl_2 and DMF (90 : 10 v/v, 40 ^oC) containing 0.2 mL of 0.1M (corresponds to 0.2 mM in 100 mL solvent) pH 4.0

acetate buffer for 72 h incubation. *Candida rugosa* lipase (CRL) showed broad substrate specificity towards amino acids as well as carbohydrates. Esterification yields were obtained in the range of 7-79%. About 29 L-prolyl (**16a-c** – **23a** and **b**, L-phenylalanyl **(24a-e – 31a** and **b)**, L-tryptophanyl **(32a-e – 39a** and **b)** and L-histidyl (**40a-e – 44)** esters of carbohydrates were prepared, of which 19 esters have not been reported before. These are: L-prolyl-D-glucose **16a-c**, L-prolyl-D-galactose **17a-c**, L-prolyl-D-mannose **18a-e**, L-prolyl-D-ribose **19a** and **b**, L-prolyl-D-fructose **20a-c**, L-prolyl-lactose **21a** and **b**, L-prolyl-maltose **22a-c**, L-prolyl-D-sorbitol **23a** and **b**, L-phenylalanyl-D-arabinose **28a-c**, L-tryptophanyl-D-mannose **33**, L-tryptophanyl-D-galactose **34**, L-tryptophanyl-D-fructose **35a** and **b**, L-tryptophanyl-lactose **36a** and **b**, L-tryptophanyl-maltose **37a-d**, L-histidyl-D-glucose **40a-e**, L-histidyl-D-mannose **41a** and **b**, L-histidyl-D-fructose **42**, L-histidyl-maltose **43a-c** and L-histidyl-D-mannitol **44**. Aldohexoses (**5-7)**, ketose (**8)** and disaccharides (**11** and **12)** showed better conversions with all the four L-amino acids **1-4**. L-Tryptophan **3** showed lesser conversion (7-70%) to esters compared to the other three amino acids (20-79%). L-Tryptophanyl-D-galactose **33**, L-tryptophanyl-D-mannose **34**, L-tryptophanyl-sucrose **38**, L-histidyl-D-fructose **42** and L-histidyl-D-mannose **44** formed only one ester. D-Glucose gave five diastereomeric esters with L-phenylalanine **24a-e**, L-tryptophan **32a-e** and L-histidine **40a-e** and the only exception being L-proline where only three monoesters **16a-c** are formed. Characterization of the isolated product esters (**16a-c** – **44**) using UV, IR, mass and NMR spectroscopy were carried out. Molecular ion peaks in mass spectrum further confirmed the formation of esters. Two-dimensional HSQCT NMR spectroscopy of the product esters (**16a-c** – **44**) gave good information on the nature and proportion of the esters formed. Nature of the products clearly indicated that

primary hydroxyl groups of the carbohydrates (*1-O-, 5-O-, 6-O-, 6′-O-* and *6,6′-di-O-*) esterified predominantly over secondary hydroxyl groups (*2-O-, 3-O-* and *4-O-*). Anomeric region in NMR clearly indicated that both α and β anomers reacted in case of D-glucose **5**, D-galactose **6**, D-mannose **7**, D-arabinose **9**, D-ribose **10**, lactose **11** and maltose **12**.

Chapter **FIVE** describes kinetic study of the esterification reaction between D-glucose **5** and L-phenylalanine **2** catalyzed by lipases from *Rhizomucor miehei* (RML) and *Candida rugosa* (CRL). Detailed investigation showed that both RML and CRL followed Ping-Pong Bi-Bi mechanism with two distinct types of competitive inhibitions. Graphical double reciprocal plots and computer simulative studies showed that competitive double substrates inhibition at higher concentrations leading to dead-end inhibition in case of RML by both L-phenylalanine and D-glucose and in case of CRL, only by D-glucose at higher concentrations leading to dead end lipase-D-glucose complex. An attempt to obtain the best fit of these kinetic models through curve fitting yielded in good approximation, the values of important kinetic parameters, RML: k_{cat} = 2.24±0.23 mM/h.mg protein, $K_{m\ L\text{-phenylalanine}}$= 95.6±9.7 mM, $K_{m\ D\text{-glucose}}$ = 80.0±8.5 mM, $K_{i\ L\text{-phenylalanine}}$ = 90.0±9.2 mM, $K_{i\ D\text{-glucose}}$ = 13.6±1.42 mM; CRL: k_{cat} = 0.51±0.06 mM/h.mg protein, $K_{m\ L\text{-phenylalanine}}$ = 10.0± 0.98 mM, $K_{m\ D\text{-glucose}}$ = 6.0 ± 0.64 mM, $K_{i\ D\text{-glucose}}$ = 8.5 ± 0.81 mM.

Chapter **SIX** describes potentiality of L-prolyl-D-glucose **16a-c**, L-prolyl-D-fructose **19a-c**, L-prolyl-D-ribose **20a** and **b**, L-prolyl-lactose **21a** and **b**, L-phenylalanyl-D-glucose **24a-e,** L-phenylalanyl-D-galactose **26a-e**, L-phenylalanyl-D-fructose **27a** and **b**, L-phenylalanyl-lactose **29a-c**, L-phenylalanyl-D-mannitol **31a** and **b**, L-tryptophanyl -D-glucose **32a-e**, L-tryptophanyl -D-fructose **35a** and **b**, L-histidyl-D-glucose **40a-e**, L-

histidyl-D-fructose **42** and L-histidyl-D-mannitol **44** as inhibitors towards Angiotensin Converting Enzyme (ACE) activity. Amino acyl esters of carbohydrates tested for ACE inhibition activity showed IC_{50} values for ACE inhibition in the 0.9 mM to 13.6 mM range. L-phenylalanyl-D-glucose (IC_{50}: 1.0±0.09 mM), L-tryptophanyl-D-fructose **35a** and **b** (IC_{50}: 0.9±0.09 mM) and L-histidyl-D-fructose **42** (IC_{50}: 0.9±0.09 mM) showed the best ACE inhibitory activities.

The present investigation has thus demonstrated the potentiality of RML, PPL and CRL to syntheses biologically and nutritionally active amino acyl esters of carbohydrates (**16a-c – 44**) using unprotected and unactivated L-amino acids and carbohydrates.

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